

## Study of Aluminum Toxicity on Photosynthetic Pigment, Soluble Sugars and Proline Contents in Two Sunflower Varieties

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### ABSTRACT

Aluminum is one of the most important heavy metals which not only can be easily absorbed by roots, but it also damages its normal function and blocks absorption of water and nutrients. According to this fact that sunflower has been widely used in industry, this study investigates the effects of aluminum toxicity on biochemical factors in two sunflower varieties including Sirena (tolerant) and Sanbero (sensitive). The study was carried out in a completely randomized design with aluminum (0, 100, 200, 300, 400, 700  $\mu\text{M}$ ) treatments and four replications in hydroponic culture. Experiments are conducted in establishment stage of the plant in four iterations at concentrations of 0, 100, 200, 300, 400, and 700  $\mu\text{M}$  of aluminum. Results of biochemical tests show that aluminum, as a heavy metal, reduces the content of photosynthetic pigments and soluble sugars. In addition, it causes toxicity in sunflower plant. Increase in the proline content of two sunflower varieties show that varieties (Sirena and Sanbero) are more vigorous against oxidative stress with low concentrations of aluminum.

**Keywords:** Aluminum, Carbohydrate, Chlorophyll, Proline, Sunflower

### INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an annual plant from composite family. This plant has big 35 centimeters flowers in diameter. It is relatively robust against soil salinity and has good growth in the range of neutral acidity (Kastro *et al.*, 2011).

Aluminum, which can be found in soil, water and air (Khavari nejad *et al.*, 2000), is the third element in terms of abundance. In case of neutral pH, aluminum is not soluble and can be found as aluminum oxide and silicate, while phytotoxic form of aluminum will be spread in soil solution and affect root and plant growth when pH decreases. The first effect of aluminum toxicity is its negative effect on root growth (Arsintescu *et al.*, 2001). Aluminum, which can be found in acidic soils, can be absorbed by roots and damages its normal function as well as limits root growth by blocking water and nutrients absorptions (Kastro *et al.*, 2011). Existence of the Aluminum in the soil solution causes aluminum hydroxide form and higher acidity of soil (Conyers *et al.*, 1990). Aluminum toxicity is the main limiting factor in crop production in acidic soils which affects above 40 percent of farmable lands across the world, especially in tropical and semitropical areas. Aluminum accumulated in root top that includes root cap and meristematic area (Silva *et al.*, 2002). The signs of aluminum toxicity are not easily detectable. In plants, the signs of aluminum toxicity are similar to the signs of phosphorus deficiency in leaves. It seems that biochemical factors are affected in sunflower because of existence of the aluminum stress. In addition, it seems that different concentrations of aluminum have different effects on sunflower. Recent studies show that resistance genotypes against aluminum in wheat, corn, sunflower, and soybean prevent it from entrance into the root top.

Some heavy metals such as cadmium, lead and mercury affect the growth and the function of plants at high concentrations. The ability of cadmium absorption is investigated in sunflower in terms of the effects on morphological and physiological characteristics (Sarhaddi *et al.*, 2011). Results showed high ability of this plant in cadmium absorption but significant effect was not found on morphological characteristics. An experiment about the effect of heavy metal of chromium on sunflower showed that protein content can be affected and decreased by chromium tension (Pirouz *et al.*, 2012). Mechanisms of aluminum tolerance in plants are composition and permeability of plasma membrane, making rhizosphere alkaline, aluminum division in vacuole, production of organic acid and secretion of phosphoric compounds (Taylor, 1991). In this research, we try to investigate the effects of aluminum toxicity on some physiological and biochemical factors in two varieties of Sirena and Sanbero in sunflower plant.

## MATERIALS AND METHODS

Initially, the seeds of two sensitive and tolerant varieties of sunflower were planted in pots containing perlite. Eight seeds were planted in each pot with the depth of 3 centimeters. Pots were watered with distilled water until seedling emergence. Then, they were watered with Hoagland solution during 2 weeks until 4<sup>th</sup> bracteoles appeared. After that, they were treated with different aluminum chloride concentrations including 0, 100, 200, 300, 400 and 700 micro molar (Feng *et al.*, 2001). After 14 days, plants were collected in order to investigate the effects of the aluminum toxicity on some biochemical factors.

### *The Measurement of Photosynthetic Pigments*

In order to measure photosynthetic pigments including chlorophyll a, chlorophyll b, and carotenoid, fresh leaves were used after exerting heavy metal stress period. The concentrations of chlorophyll a, chlorophyll b and carotenoid were calculated by lichtenthaler method (1987).

In this method, absorption rate of photosynthetic pigments at wavelengths of 663, 645, 470 and 659 nanometers were obtained with spectrophotometer and measured with Lichtenthaler equations as follows :

$$\text{Chla} = 12.21(A_{663}) - 2.81(A_{645})$$

$$\text{Chlb} = 20.13(A_{645}) - 5.03(A_{663})$$

$$\text{Car.} = (1000A_{470} - 3.27[\text{Chla}] - 104 [\text{Chlb}]) / 227$$

$$\text{chl(a+b)} = \text{chla} + \text{chlb}$$

#### *Proline Measurement*

In order to measure the proline content in plant tissues, Bates method( 1973) was used. 0.04 grams of plant tissue were ground into 1.7 milliliters of solphosalisilic acid (3%). Produced extracts were transferred into Eppendorf 5415R. They were under centrifugal force for 20 minutes ( r/m?) in Eppendorf 5415R (10000 gr). In next stage, 1.0 milliliter of the extract was taken from each Eppendorf and transferred in 10milliliter test tube. Then, 1.0 milliliter of ninhydrin acid and 1.0 milliliter of pure acetic acid were added in test tubes. Each tube was covered by aluminum foil and test tubes were heated at 100 C for 1 hour. Heating caused reaction between ninhydrin and proline. Then, test tubes were kept for 1 hour in room temperature. Two milliliters of toluene were added to each tube. They were shaken by shaker for 2 minutes. The tubes were kept at laboratory temperature for 30 minutes. During this time, two organic phases (pink in upper and colorless in lower part) were formed in each tube. One millimeter of upper part containing toluene and proline was separated and the absorption of each sample at wavelength of 520 nanometers was read by spectrophotometer system. Finally, the amount of the proline was calculated.

#### *Carbohydrate Measurement*

In order to measure the amount of carbohydrate, Kochert method (1978) was used. For experiment, 0.1 grams of dry material were poured in the test tube. Then, 10 milliliters of ethanol (%70) were added to it. It was put into refrigerator for a week. After a week, 0.5 milliliters of upper solution were taken and its volume reached 2 millimeters with distilled water. Then, one millimeter of phenol 5% and 5 milliliters of heavy sulfuric acid were added, respectively. A yellow solution was produced. It gradually changed into light brown solution. This solution was kept at laboratory temperature for 30 minutes in order to obtain the final color. Intensity of the produced color was read by Spectrophotometer at wavelength of 485 nanometers.

#### *Statistical Analysis*

Differences in the plants' physiological parameters under heavy metal effects were compared using ANOVA with means separation by Duncan's test using SPSS 18 software at a significance level of  $p < 0.05$ .

## RESULTS

### *Photosynthetic Pigments Amount*

In this research control sample had the least effect and application of 700  $\mu\text{M}$  of aluminum had the most effect on chlorophyll a, chlorophyll b and total chlorophyll. Results from the measurements of chlorophyll a, chlorophyll b and total chlorophyll are shown in Figures 1, 2 and 3, respectively. Increase in the amount of aluminum concentration decreases the contents of chlorophyll a, chlorophyll b and total chlorophyll in both varieties of Sirena and Sanbero. This decrease was significant in Sanbero for chlorophyll a, chlorophyll b and total chlorophyll at concentrations of 300, 400 and 700  $\mu\text{M}$  of aluminum. Moreover, it was significant in Sirena for chlorophyll a at concentrations of 300, 400, and 700 micro molar and for chlorophyll b and total chlorophyll at concentration of 400 and 700  $\mu\text{M}$  of aluminum compared with control group.

### *Carotenoid Content*

Figure 4 shows results from analysis of variance for aluminum tension on carotenoid in two varieties of Sirena and Sanbero. Results showed that carotenoid content was decreased in both varieties with aluminum treatment. This decrease is significant for Sanbero at concentration of 700  $\mu\text{M}$  and for Sirena at concentrations of 300, 400 and 700  $\mu\text{M}$  of aluminum in comparison with control group.

### *Carbohydrates Content*

Results from the measurement of soluble carbohydrates in two varieties of Sirena and Sanbero under aluminum stress are shown in Figure 5. Results showed that concentrations of 100 and 200 micro molar of aluminum increased the content of soluble carbohydrates in both varieties of Sirena and Sanbero. This increase was statistically significant in comparison with the control group.

### *Proline Content*

Results of analysis of variance for the effects of aluminum saturation on proline contents in two varieties of Sirena and Sanbero are shown in Figure 6. Results showed that proline content increased at concentrations of 200 and 300  $\mu\text{M}$  of aluminum in Sanbero and at concentrations of 100, 200 and 300  $\mu\text{M}$  of aluminum in Sirena. This increase was not significant in comparison with the control group.

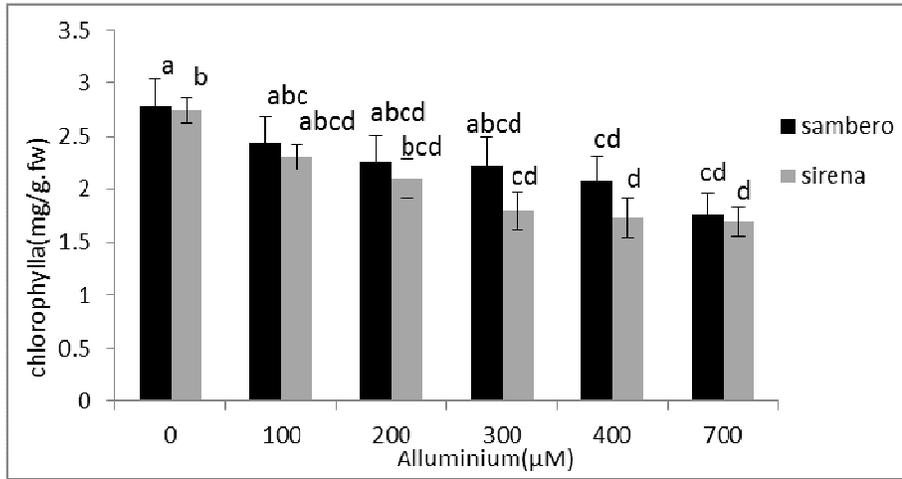


Figure 1. Effects of aluminum toxicity on the content of chlorophyll a. The same letters show no significant difference at  $p < 0.05$ .

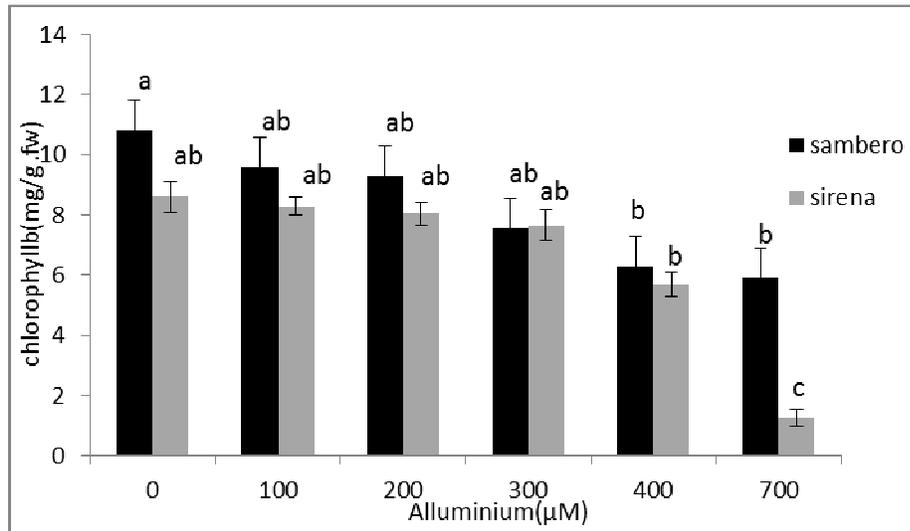


Figure 2. Effects of aluminum toxicity on the content of chlorophyll b. . The same letters show no significant difference at  $p < 0.05$ .

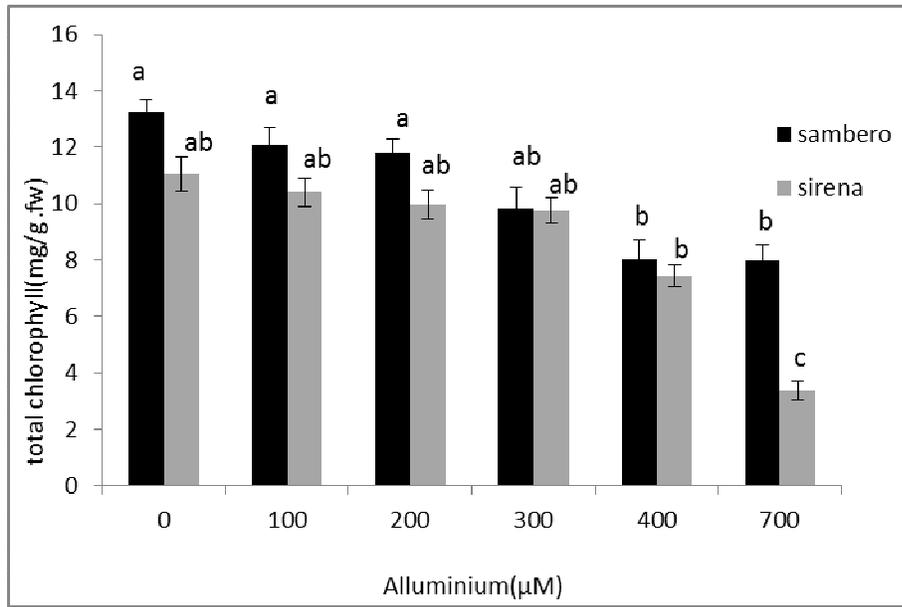


Figure 3. Effects of aluminum toxicity on the content of total chlorophyll. . The same letters show no significant difference at p<0.05.

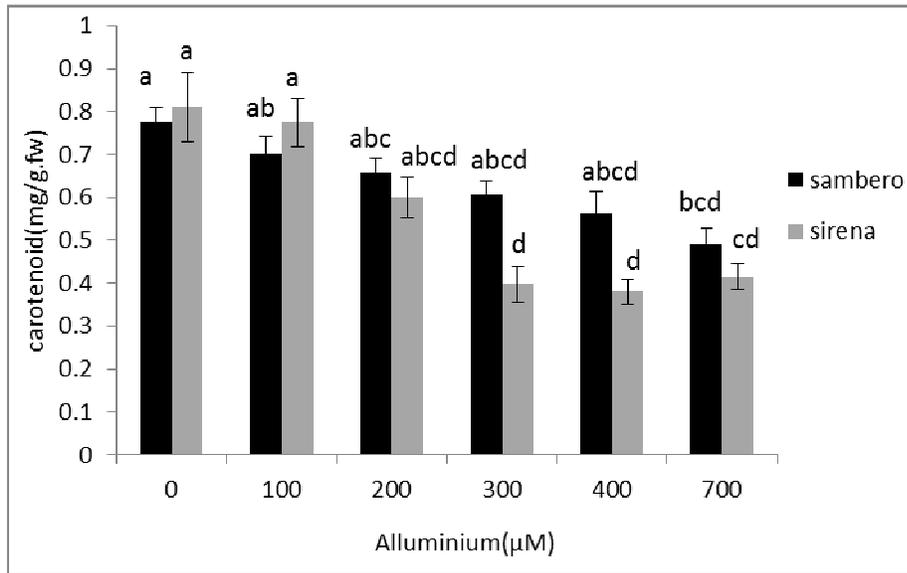


Figure 4. Effects of aluminum toxicity on the content of carotenoid. The same letters show no significant difference at p<0.05.

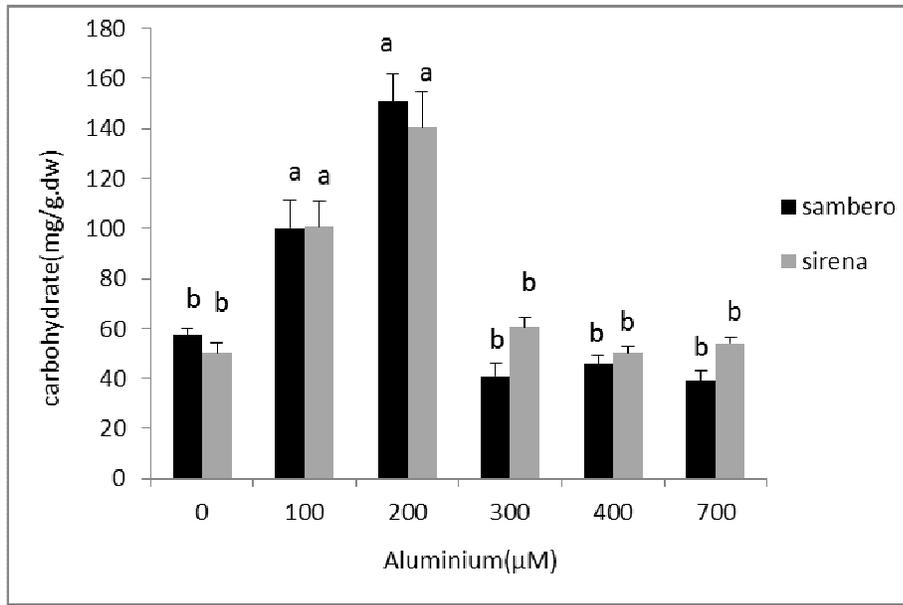


Figure 5. Effects of aluminum toxicity on the content of carbohydrate. The same letters show no significant difference at  $p < 0.05$ .

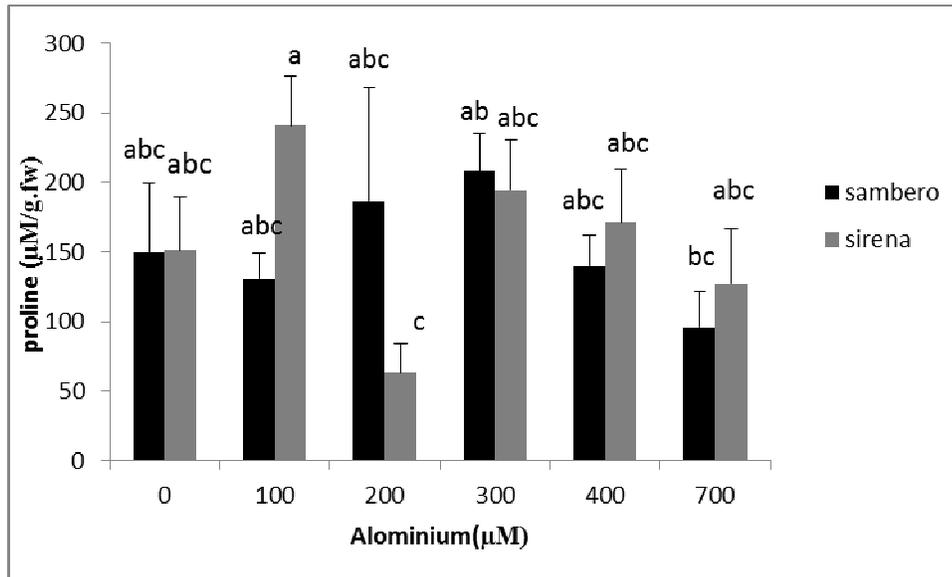


Figure 6. Effects of aluminum toxicity on the content of proline. The same letters show no significant difference at  $p < 0.05$ .

## DISCUSSION AND CONCLUSION

### *Photosynthetic Pigments*

All heavy metals significantly lowered the leaf contents of the photosynthetic pigments (Aldoobie and Beltagi, 2013). Also in this research, aluminum treatment decreased the contents of the pigments of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in the two varieties of Sirena and Sanbero. This decrease was statistically significant compared with control group. The decrease of pigment levels, as a result of heavy metals, has been found in many plants (Van *et al.*, 1990). According to results from broad bean, aluminum toxicity causes the reduction of root respiration and photosynthesis (Arsintescu *et al.*, 2001). The content of photosynthetic pigment is decreased, because of the destruction of chloroplast structure, photosynthetic system and chlorophylls photo oxidation, the destruction of the pre-material of chlorophyll synthesis and the inhibition of chlorophyll biosynthesis. One of the causes of photosynthetic pigments reduction during the stress of heavy metals is the production of oxygen active species that cause breakdown and decrease pigments. During different stresses, existing chlorophyll in chloroplast is broken down and thylakoid structure disappeared (Rout *et al.*, 2001).

### *Carbohydrates Content*

In this research, the content of soluble sugars in sunflower were increased using aluminum treatment. This increase was statistically significant at concentrations of 100 and 200  $\mu\text{M}$  for the varieties of Sirena and Sanbero. This result is similar to the report obtained from aluminum treatment on bean that causes increase in soluble sugar in leaves (Khavarinejad *et al.*, 2010). The increase of aluminum augments the content of carbohydrates in rice (Prasad, 1995). Carbohydrates accumulation affects the maintenance of cellular membrane and osmotic regulation (Sato *et al.*, 2004). The increase of aluminum concentration causes problem in intracellular water balance and subsequently, extra cellular changes occur in cellular organelles such as tonoplast and enzymes in the direction of sugars metabolism. With the increase of aluminum concentration, the activity of the invertase enzyme is decreased (Prasad 1995). Decrease of transferred water to leaves and the accumulation of this heavy element in cells, the content of carbohydrates increases as a mechanism for the maintenance of osmotic potential under aluminum stress.

### *Proline Content*

In order to investigate the sunflower response to aluminum toxicity, proline content, as one of the most important materials which is adaptable to environmental tensions, was evaluated. Results showed that increase in the amount of the aluminum in the shoots of sunflower augments proline content. Proline accumulation is one of the adaptive responses in different species of plants to stress from heavy metals. In addition, research on tomato has shown that tension from heavy metals increases proline content (Lari Yazdi *et al.*, 2000). In this research, proline increase can moderate aluminum tension by its osmolyte and antioxidant characteristic.

Research on the effect of aluminum on soybean has shown that aluminum can augment proline content (Liu *et al.*, 2004). Also, the effect of other heavy metals on sunflower have shown to increase significantly the proline content (Lari Yazdi *et al.*, 2000; Pirouz *et al.*, 2012).

### CONCLUSIONS

The results of biochemical analyses show aluminum, as a heavy metal, decreases photosynthetic pigments and soluble sugars while increases proline and causes toxicity in sunflower.

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